Review of Environmental Morphine Identifications: Worldwide Occurrences and Responses of Authorities

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Opium poppies grow wild worldwide, and testing for morphine is now highly sensitive. Currently, many authorities worldwide do not pursue urinary morphine concentrations of <100 ng/ml. This is because such low urinary morphine concentrations are likely to be environmental morphine identifications (EMIs) and are also unlikely to be associated with pharmacological responses. Authors' addresses: Maxwell Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546 (Camargo, Karpiesiuk, Tobin); e-mail: fccama2@uky.edu (Camargo); U.K. Livestock Disease Diagnostic Center, Lexington, KY 40512 (Lehner); Florida Horsemen's Benevolent and Protective Association, Miami, FL 33024 (Stirling); Department of Pharmacology, Trinity College, Dublin, Ireland (Kavanagh); and Connolly's Redmills, Goresbridge, Kilkenny, Ireland (Brennan, Dowling).

1. Introduction

Morphine, the original μ agonist opiate, is derived from the juice of the opium poppy, *Papaver somniferum*. Man has long cultivated *P. somniferum* and facilitated its spread around the world. Morphine has also been detected in rice, beer, lettuce, hay, and human and bovine milk.

In horses, morphine and related opiates stimulate locomotor activity, suppress pain, and seem to prolong endurance. These are potentially useful actions in racing horses, and opiates have a long history of improper use in racing.

In the early 1990s, sensitive enzyme-linked immunosorbent assay (ELISA) tests for morphine and other opiates were introduced, abruptly terminating patterns of opiate abuse that had been in place for 100 yr. However, these tests have also yielded sporadic sequences of low level (<100 ng/ml) morphine identifications in post-race urines around the world.

These low-level morphine identifications first became a problem in Australia, where the morphine was traced to wild *P. somniferum* or *segiterum*. At the same time, low concentration morphine identifications became a problem in Hong Kong, which imported much of its feedstuffs from Australia. The solution adopted in Hong Kong, apparently on an interim basis, was a "reporting limit" for morphine of 100 ng/ml.

NOTES

Fig. 1. Putative *P. somniferum*, yielding morphine, growing wild outside a Dublin, Ireland research laboratory.

A 100 ng/ml reporting limit for morphine is well supported in the scientific literature. The intravenous "No Effect Dose" for locomotor responses to morphine in horses is ~0.1 mg/kg or 50 mg for a 1000-lb horse. This dose yields peak urinary concentrations of morphine glucuronides of ~22,000 ng/ml; therefore, the dose to produce a urinary concentration of 100 ng/ml is ~250 μg, a dose highly unlikely to produce a pharmacological effect. Another consideration is that oral morphine is unlikely to be more than ~20% bioavailable in the horse, further reducing the probability of a pharmacological response from environmental (oral) exposure to morphine.

These conclusions are independently supported by a recent and very conservative pharmacokinetic/pharmacodynamic analysis, which suggested that the "irrelevant" concentration of morphine in equine urine is ~80 ng/ml.

Low concentration identifications of morphine have also been observed in a number of U.S. jurisdictions, leading Ohio and Louisiana to introduce urinary morphine "reporting limits" of 50 and 75 ng/ml, respectively. In late 2002, in the United Kingdom and Ireland, a sequence of low concentration morphine identifications associated with manufactured feed led the British Jockey Club to introduce a 50 ng/ml reporting level for morphine.

In summary, there is a worldwide propensity for sporadic low level environmental morphine identifications (EMIs), apparently from botanical sources of morphine. The most practical and widely used solution has been to set a reporting level for morphine of 50–100 ng/ml, which effectively eliminates the administrative and public relations problems associated with very low-concentration EMIs.

2. Background

The word "opium" is derived from the Greek word for juice, and raw opium is the congealed juice of the opium poppy, *P. somniferum* (Fig. 1).

The juice of *P. somniferum* contains at least 20 alkaloids,[1] and the most pharmacologically useful and widely used of which is morphine. Evidence for the use of opium/morphine for medicinal purposes occurs in the earliest human records, and morphine is still cultivated commercially throughout the world. Some morphine is grown under license for the pharmaceutical industry; some, presumably much more, is grown "off-license" for other markets, and an unknown but possibly substantial fraction of the morphine growing in the world today may be ornamental/feral/wild growth on the part of *P. somniferum* itself and/or its various relatives and/or variants.[2,3]

Morphine produces its pharmacological effects by interacting with μ-opioid receptors. In humans, opioids, including morphine, classically produce analgesia and drowsiness, affect mood, and alter respiratory, cardiovascular, and gastrointestinal functions.[1] The effects of opioids in a variety of systems, including the central nervous system (CNS), are caused by the wide distribution of opioids and their receptors both in the brain and in the periphery.[1]

Peak blood concentrations of morphine found after oral administration of morphine are from five- to seven-fold less than those obtained after its parenteral administration,[4] and thus, the effect of any given dose is less after oral administration than after parenteral administration. Morphine has a significant first-pass metabolism in the liver, and therefore, the effect of a given dose is generally considerably less after oral than parenteral administration. The major urinary metabolites are morphine-3-glucuronides and morphine-6-glucuronides; relatively little morphine (~2–10%) is excreted unchanged in urine.[5]

In horses, morphine produces excitement, increased locomotor activity, and alertness, as described in the late 1970s by Combie et al.[6] These workers also showed that when administered at a dose of 0.1 mg/kg, IV, morphine elimination followed a three-compartment open system with a terminal serum half-life of 87.9 min and a urinary half-life of 101.1 min. Morphine was detected in serum for up to 48 h and in urine for up to 144 h. Urinary "total" morphine peaked at 2 h after dosing at an average of 21,894 ng/ml, and it decreased thereafter. Morphine was detected in hydrolyzed and unhydrolyzed urine up to 144 h after dosing (Fig. 2).[7]

In the early 1990s, highly sensitive ELISA tests for morphine were introduced into racetrack testing,[8,9] and instrumental confirmatory methods also became highly sensitive.[10,11] These tests made it readily possible to detect[9] and confirm[11] concentra-

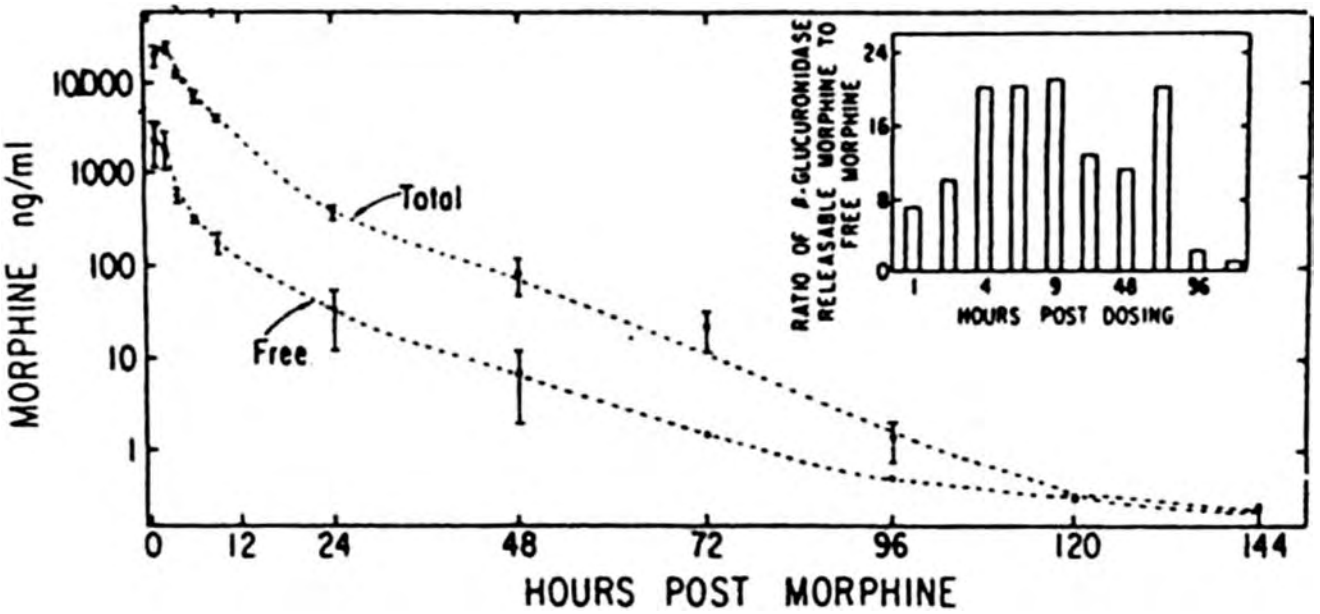

Fig. 2. Urine morphine concentration after administration of 0.1 mg/kg, IV to horses.[10]

tions of morphine as low as 10 ng/ml or less in post-race urines.

Simple mathematics shows that if a dose of morphine of 50 mg/horse yields a urine concentration of >20,000 ng/ml urine, as little as 25 μg/horse will yield a urinary concentration of 10 ng/ml. However, the probability of a pharmacological effect resulting from such a small dose of morphine is, for all practical purposes, zero.

More recently, Kollias-Baker and Sams[12] reported a study in which 10, 5, and 1 g of poppy seeds were administered orally to horses. The horses administered 10 g of poppy seed received 732 μg of morphine; the ones administered 5 g received 366 μg, and finally, the ones administered 1 g received 73.2 μg. No behavioral changes or pharmacological effects were observed in any of the horses.

In these experiments, urinary concentrations of morphine were 213 ng/ml after the 732-μg dose, 119 ng/ml after the 366-μg dose, and 27.6 ng/ml for the 73.2-μg dose, which agrees with our work from 25 yr ago.[7,10] Peak urine concentrations occurred within 4 h of administration. Morphine was detectable by ELISA 24 h after administration in all urine samples of the horses dosed except for one; morphine was not detected in the urine of one horse dosed 73.2 μg of morphine by means of ingestion of 1 g of poppy seeds.

A similar study had been performed by Ginn et al.,[13] where 2 g of poppy seeds were administered with feed twice a day for 3 days to three horses. Urinary excretion after poppy seed administration showed maximum morphine concentration to be up to 120 ng/ml. The morphine concentration declined below their limit of detection at 35–40 h after the last administration of poppy seeds.

Poppy seeds are far from being the only dietary sources of morphine. Trace amounts of morphine, between 280 and 1400 pg/kg, have been detected in bread, rice, vegetable soup, milk, and beer. Higher concentrations (5.6 μg/kg dry weight) have been reported in different types of lettuce.[a] Hazum et al.[14] have found morphine in hay and lettuce at concentrations between 2 and 10 ng/g dry weight, and they "postulate that morphine may be a ubiquitous constituent of plant-derived foods."[14]

Since the introduction of the ELISA[9,15–17] tests in the early 1990s, there have been sporadic low-concentration identifications (<100 ng/ml) of morphine in post-race urine samples in horses racing around the world. Surprisingly, a considerable proportion of these identifications are associated with areas in which morphine has, at one time or another, been cultivated commercially. However, this presentation begins with a report of identifications in Ireland, a country with, to our knowledge, no history of organized commercial cultivation of morphine.

3. Ireland

Ireland, where morphine has never been grown commercially, has recently seen an incident of nine low-level (21–46 ng/ml) morphine "identifications" associated with manufactured racehorse feed. These unexpected findings greatly increased the level of interest in the "wild" opium poppies growing in Ireland. In this regard, our colleagues, Dr. Kavanagh, Dr. Scott, and Dr. Lambert of Trinity College, soon identified significant numbers of "wild" opium-containing poppies growing at various locations in Ireland (Figs. 3 and 4) as well as outside of their Dublin laboratory.

Fig. 3. Putative *P. somniferum*, yielding morphine, growing wild in rural Ireland.[18]

Our colleagues soon showed that these sporadic "wild" sources of morphine have the potential to yield more than sufficient opium/morphine to give rise to low-level identifications of morphine in horse urines.[18] In assessing the significance of these Irish and other similar findings that we will set forth, we must bear in mind that man has been cultivating and using *P. somniferum* for at least 5000 yr, and it is only within the last 100 yr that its cultivation and use have been closely regulated. For most of recorded history, man has cultivated variants of *Papaver* and has carried it with him throughout the old world[19] and to the New World and Australia long before its cultivation was regulated. As such, the finding of wild opium-containing plants related to *P. somniferum,* quietly growing at diverse locations throughout the world, should come as no surprise.[2,3]

We might also note that if the sources of such low-concentration morphine identifications in horses are indeed of local and botanical origin, then one might reasonably expect that these findings would be characteristically seasonal. This possibility may have been observed (it seems to have occurred in California in 2000 and the United Kingdom in 2002) and should be considered during the evaluation of incidents or episode(s) of low-concentration morphine identifications.

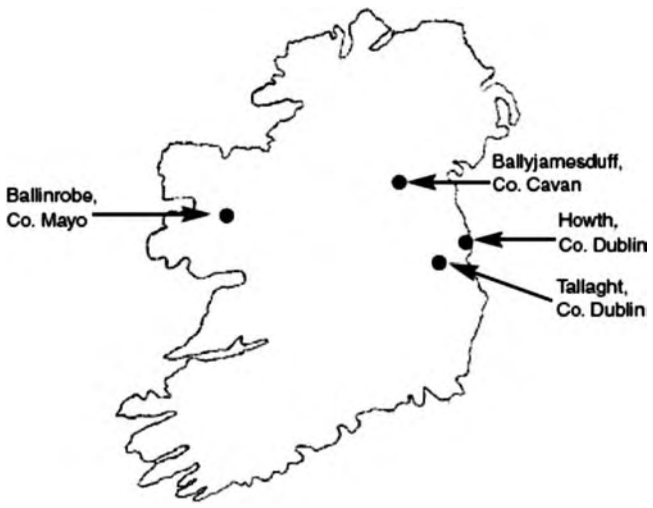

Fig. 4. Geographic locations at which putative *P. somniferum* yielding morphine have been located growing wild in Ireland.[18]

4. United Kingdom

The incident of EMIs in a commercial Irish racehorse feed, apparently from a commercial feed ingredient, also gave rise to a number (~30) of positive identifications for morphine in post-race urine samples from horses racing in the United Kingdom in late 2002 and early 2003. One outcome of these events was that the English Jockey Club, in early 2003, raised the reporting level ("threshold") for morphine (as morphine glucuronides) in post-race urines in horses from the previously estimated 10 ng/ml limit of detection (LOD) to a current 50 ng/ml LOD.

This was an unusual move, and the English Jockey Club only adjusted their morphine reporting level or "threshold" after the repeated assurance of their Chief Veterinarian, Dr. Peter Webbon, that a concentration of 50 ng/ml of morphine, as morphine glucuronides, in a post-race urine would not, or could not, under any circumstances be associated with a performance effect on the racing horse at the time of the race in question.

Additionally, since the introduction of this new reporting level, the Jockey Club authorities, in a letter dated October 28, 2004, noted that "since June 2004 there have been 17 instances where urine samples have indicated the presence of morphine at low levels." However, they were all below 50 ng/ml, so they were reported as negatives. It started with 2 or 3 instances/mo, but in September, there were 8; therefore, the two feed companies that were supplying feed to the trainers concerned were approached. Samples of feed were taken for analysis, but the Horseracing Forensic Laboratory (HFL)[b] "did not find any traces of morphine . . . The Jockey Club has not had any reports from HFL for low levels of morphine for the last four weeks."

We also specifically draw attention to the fact that the Jockey Club letter notes that although these horses were testing "positive" for morphine, analysis

of samples of the feedstuff was unable to definitively identify morphine.

We might add that, based on some earlier research from our group[11] and a review of the scientific and regulatory literature,[20,21] Dr. Webbon was apparently on very safe grounds when he concluded that these concentrations of morphine would not be associated with any effect on the racing performance of a horse.

5. France

In France, in the 1990s, opium poppies were cultivated under license as a French domestic source of medicinal morphine. These opium poppies were then dried in commercial hay drying equipment during their processing. Not unexpectedly, opium poppy residues in these hay driers apparently "cross-contaminated" later shipments of agricultural hay dried in the system. Some of this hay containing trace amounts of morphine was then fed to racehorses, which then yielded morphine "positive" racehorse urines. This gave rise to a number of low-concentration morphine glucuronide identifications in post-race urines in France.

With regard to the French racing authorities, we do not know what the in-house reporting level, if any, for morphine in France is; however, it seems as though the French authorities had, at one time, a problem with low-level concentrations of morphine in their post-race urine samples, and our understanding is that the morphine ELISA test developed at the University of Kentucky was officially recognized as a screening test for morphine in French equine feedstuffs.

6. Australia

The opium poppy has been extensively cultivated in Australia, apparently in Tasmania, as a source of medicinal morphine. There also seems to be a related opium-containing poppies (*P. setigerum*) growing wild in Australia. These feral/wild opium-containing poppies have given rise to identifications of morphine in post-race urines in Australia since at least the mid-1990s. In this regard, at least one Australian jurisdiction had an "in-house" unpublished limit of detection/reporting level for morphine of 100 ng/ml, and personal communication[c] with this authority commented on the difficulty of completely excluding sources of morphine from horsefeed under Australian conditions.

These comments on the technical difficulty encountered in trying to ensure that Australian feed is free of morphine are of interest with respect to the related comments set forth in the British Jockey Club letter referenced above.

7. Hong Kong

As set forth above, low-level morphine identifications are not uncommon in Australia, and at one time, the Hong Kong Jockey Club, which imports much of its hay/fodder from Australia, had an "in-house" unpublished threshold for morphine in post-race urines of 100 ng/ml.

8. United States

In the United States, such low-concentration identifications of morphine have been reported in California, Pennsylvania, Florida, Louisiana, and Ohio (from an unidentified source).

Ohio

The outcome in Ohio was the creation of a reporting level for morphine in post-race urines of 50 ng/ml, as set forth below in the excerpt from The National Horsemen's Benevolent and Protective Association Proposed Policy on Drug Testing and Therapeutic Medication.[22]

Louisiana

The outcome in Louisiana was a published threshold for morphine of 75 ng/ml in post-race urines, as set forth below in the excerpt from The National Horsemen's Benevolent and Protective Association Proposed Policy on Drug Testing and Therapeutic Medication.[22]

An Unnamed Southeastern State

It has been communicated personally to one of the authors[d] that an unnamed southeastern racing state has an in-place unpublished reporting limit for morphine, as morphine glucuronides, of 100 ng/ml.

Pennsylvania and California

Although both Pennsylvania and California have reported low (<50–100 ng/ml) identifications of morphine and there are suggestions that at least some of the California identifications have been seasonal and presumably botanical or environmental in origin, these states have not, to our knowledge, introduced any published or in-house reporting levels or thresholds for morphine in post-race urines.

With regard to the potential for EMIs in California, it is of interest to note that a California website (http://www.calflora.org)[2] refers to *P. somniferum* growing wild in four counties.

Although the state of California has not yet introduced a reporting level for morphine in post-race urines, the California Horse Racing Board (CHRB) has recently ruled in favor of trainers with low-level morphine-positive horses. This was the case of trainers Bob Baffert, a three-time Kentucky Derby winner, and Jesus Mendoza. These trainers had horses that presented low levels of morphine in their post-race urine in the year 2000 at Hollywood Park. Both horses presented morphine urine concentrations <100 ng/ml. On January 10, 2002, the CHRB dismissed Mendoza's case,[23] ascribing the findings to environmental contamination. On March 24, 2005, following the recommendation of an administrative law judge, the CHRB sustained Baffert's appeal and dismissed the complaint,[24] also invoking the likelihood of EMIs in this case. Apparently,

during the spring of 2000, 13 of 95 urine samples tested at the same laboratory were suspect for opioids,[24] a finding which is consistent with seasonal EMIs.

Washington

The state of Washington has recently introduced a threshold/reporting level for morphine of 50 ng/ml of urine.[25]

9. The National Horsemen's Benevolent and Protective Association Proposed Policy on Drug Testing and Therapeutic Medication

As set forth above, low-concentration identifications of morphine glucuronides have occurred around the world, and a number of jurisdictions now have formal published "thresholds" and "regulatory limits" or in-house "regulatory limits" for morphine. The following excerpt is taken from the National Horsemen's Benevolent and Protective Association's Proposed Policy for Drug Testing and Therapeutic Medication that was published in the January 2003 issue of the Journal of Equine Veterinary Science.[22]

> "Morphine Glucuronides
> Target Analyte: Morphine
> Threshold/Regulatory Limit: 100 ng/ml in urine
> Three thresholds/regulatory limits for morphine glucuronides, the major urinary metabolites of an Association of Racing Commissioners International class 1 substance, a not uncommon addition to human foodstuffs as poppy seeds and also a potential environmental contaminant, are in place in the United States. The threshold/regulatory limit in one unidentified American jurisdiction is 100 ng/ml, and it is also under review in another. In Louisiana, it is 75 ng/ml; a slightly lower (50 ng/ml) limit is in place in Ohio. This threshold/regulatory limit is also under review in more than one jurisdiction. These thresholds/regulatory limits are well supported by more recent research from the Horseracing Forensic Laboratory (HFL) in England, which shows urinary concentrations of 110 ng/ml after administration to horses of 2-gram doses of poppy seeds containing 3 μg of morphine per dose. These thresholds/regulatory limits are dramatically lower than the 2,000-ng/ml 'cut-off' in place in human workplace medication testing.
>
> Withdrawal Time Guideline: No withdrawal time guidelines, since these are neither relevant nor applicable to dietary and environmental substances/contaminants."[22]

In summary, the regulatory limit in urine of 100 ng/ml of morphine as morphine glucuronides, proposed by the National Horsemen's Benevolent and Protective Association, is an extremely conservative limit; it is apparently in place in a number of jurisdictions worldwide, and there is no possibility whatsoever of a performance effect being associated with these levels of morphine in a post-race urine sample. As such, this proposed reporting limit is well supported by published scientific work and current and evolving international regulatory practice.

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